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Purpose: To ligate the purified vector + insert and transform with appropriate controls.

Ligation Rx:

				Tested
Vector ~ 100ng/λ	= 1 μl	1 : 1 "	1	Vector alone
Insert ~ 25ng/λ	= 4 μl		2	" + insert w/o any purification
5x buffer	= 4			
Ligase T4	1		3	Taq 1.5
H_2O	10			
	20 μl at 25°, 3 hrs.		4	" 2
			5	" + DV 1.5
			6	" " 2

Transformation using DH5α Max eff. cells.

Normal protocol used 2.5 μl of ligation Mix / transformation 50 μl cells.

Final volume after adding SOC ~ 500 μl

plated 25, 50 & 100 μl of each

Control diluted to 1:10 and plated 25, 50 & 100 μl.

normal transformation efficiency 1.5×10^9

Vector only — few blues because of the contamination; no whites.

Vector + insert — ligated w/o any purification, lots + lots of colonies; transformation quite efficient.

Taq + D.V } 2 mM Mg — didn't work
Taq alone }

Taq " } 1.5 mM Mg — Very few colonies in 25 μl + 50 μl; 100 μl slightly better. DV Deep Vent
Taq + D.V } for ~~better~~ two than Taq alone, However, # are so few to make a call

— all purified gave low efficiency of transformation compared to un " — Vector + insert

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Read & Understood by me, [signature] Date 12/19/94

Invented by

Recorded by K. Sitaraman Date 12/13/94

[redacted]